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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 offspring. 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.

Key words: chorus, coordination, duets, lemurs, singing, synchrony

The ability to coordinate vocal emissions is universal in humans (Stivers et al. 2009; Henry et al. 2015). Usage of strikingly diverse languages commonly present turn-taking, which also sets early in ontogeny (Stivers et al. 2009; Casillas et al. 2016). This evidence raises the question whether the ability to coordinate our utterances has evolved under particular pressures or it was already present in the last common ancestor we had with other species (Levinson 2016). Studies on birds, insects, amphibians, and mammals showed that also animals produce vocal signals in which different individuals emit in a coordinated manner. The common feature in animal's choruses is the temporal organization of participants' contribution to favor or avoid overlap (Ravignani et al. 2014). This mechanism

has evolved independently in species in which the mutual influence in the timing of signals involves an interaction among emitters driven by a complex short-scale timing behavior (Geissmann 2002; Takahashi et al. 2013). For instance, duetting between the sexes in the broad-winged bush katydid *Scudderia pistillata* showed rhythmic and synchronization abilities. In this species, the number of ticks female produce, as well as the timing of her response, depends on the number of pulses generated by the male (Villarreal and Gilbert 2013). Studies on tropical songbirds suggested that the evolution of coordinated resource-defense signals may be driven by ecological conditions that favored sedentary lifestyles and social stability (Logue and Hall 2014). In fact, males and females of duetting species usually establish long-lasting bonds, live in forested habitats, and show a territorial behavior (Thorpe 1963).

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

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

The indris, which are the only lemur species that produce songs, were indicated as good candidates for further investigations of the evolution of rhythmic abilities. Their songs exhibit turn-taking between individuals of different sexes and a variable degree of overlap between group members (Gamba et al. 2016). They produce songs that differ in their acoustic structure between contexts. The songs may serve to inform the neighboring groups about the occupation of the territory, to resolve territorial fights during a confrontation between different groups, and have a cohesion function (Pollock 1986; Torti et al. 2013). The indris' songs are composed by a long sequence of vocalizations that usually starts with a harsh emission ("roar"), followed by a series of slightly frequency modulated units ("long notes"). There is then a series of units organized in phrases with a descending frequency pattern (descending phrases [DPs], Thalmann et al. 1993; Sorrentino et al. 2013) composed of 2–6 units. The indri's vocal behavior provides a model system for studying the evolution and production of complex rhythmic signals that involve input from multiple individuals (Gamba et al. 2016). Most of the previous research has concentrated on the function of duets (Geissmann 2002; Clarke et al. 2006), but few studies have investigated rhythmic and coordination abilities in non-human primates. Our first aim was to examine rhythmic features in paired adult indris to understand which factors, and to what extent, could influence the rhythmic song's structure and its variation. We hypothesized that the indris' song output would show variability related to different variables, depending on the duration of the song, the number of singers, and the individual contribution per singer. Our second aim was to define a possible scenario to understand how the synchronization of utterances is achieved. The work by Gamba et al. (2016) showed that the indris' rhythmic characteristics are highly sexually dimorphic. They also found an effect of the adult male singing on the adult female output on most songs. A recent paper by Torti et al. (2017) showed that the females' song features are less genetically constrained than those of males, in fact they found that similarity of both temporal and frequency parameters of DPs significantly correlate with genetic distance only for males, whereas for females this results 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 20 m 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
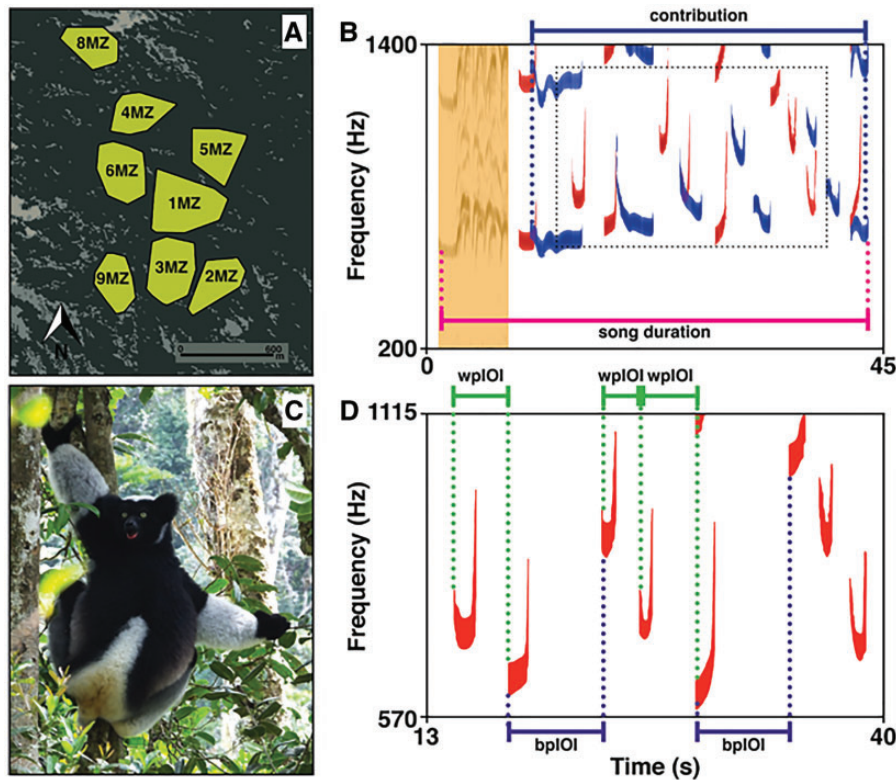
Figure 1. (A) Map of the study area in the Maromizaha Forest. Minimum Convex Polygons (MCP) generated with ArcGIS 9.1 (Environmental System Research Inc.) correspond to 2016 home range of the study groups. Group ID is reported onto each MCP. (B) Spectrogram of the indris' song generated using Praat. 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 highlighted 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.

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").

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 × 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).

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.

Table 1. Summary of group ID, N of recorded songs per group, individual ID, and sex of the individuals of the social groups considered

Group ID	N songs	Individual	Sex
1	23	Jery[a]	M
		Bevolo[a]	F
		Berthe	F
2	19	Fotsy[a]	M
		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[a]	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[a]	M
		Mika	F
		Zafy	M
9	11	Emilio[a]	M
		Sissie[a]	F
		Dosy	F

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.

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).

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

Table 2. Influence of the fixed factors on contribution, phonation, wpIOI, and bpIOI

Factors	Estimate	SE	df	t	P	Factors	Estimate	SE	df	t	P
Contribution						**Phonation**					
(Intercept)	1.197	0.135	a	a	a	(Intercept)	0.280	0.120	a	a	a
SexM[b,c]	−0.121	0.038	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	233.753	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	−12.697	**<0.001**	nDPs	0.119	0.020	228.190	6.013	**<0.001**

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.

Table 3. Influence of the fixed factors on male's and female's inter-onset intervals

	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**

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.

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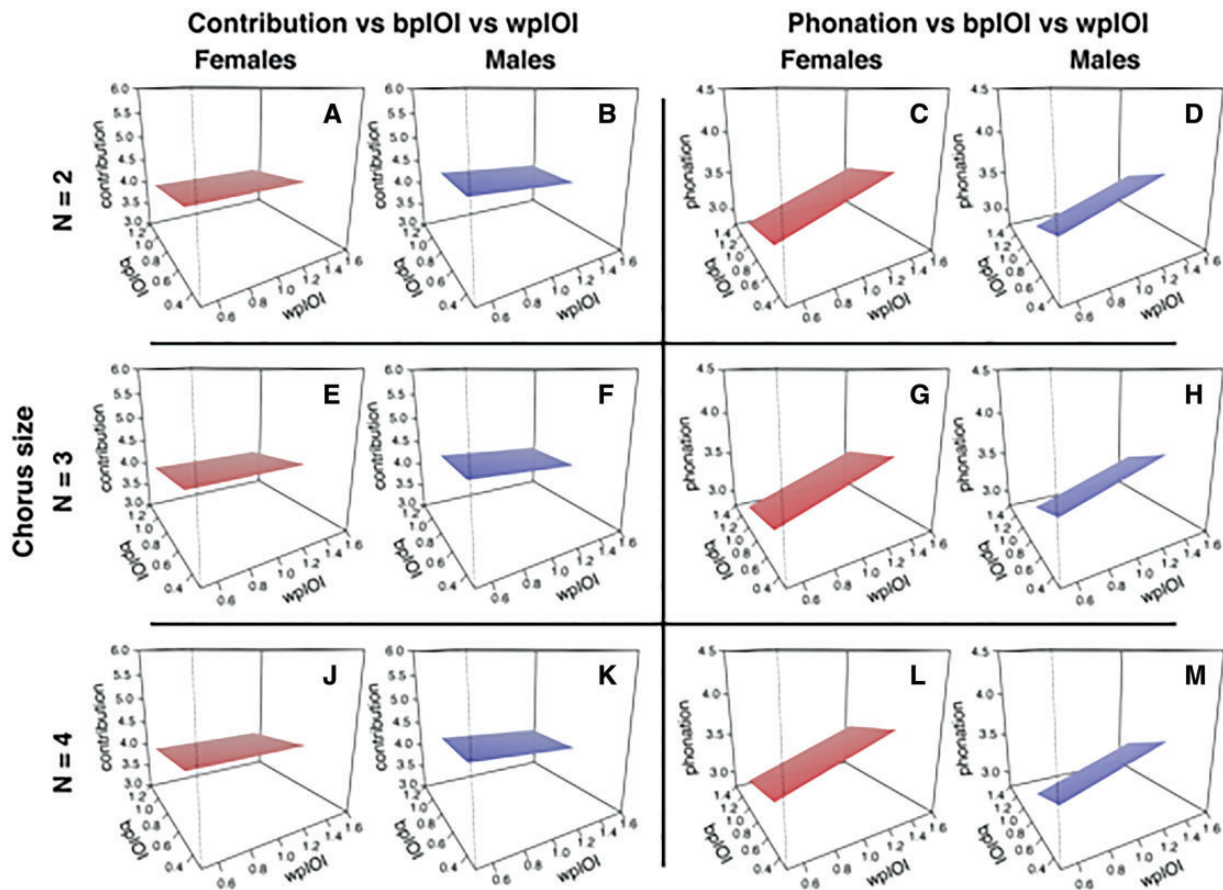
Figure 2. Interaction between contribution and phonation with bpIOI and wpIOI during the indris' song ($N = 119$). The individual mean durations are 2.698 ± 0.705 s for the wpIOI, 2.219 ± 0.330 s for the bpIOI, 69.768 ± 32.452 s for contribution and 28.416 ± 11.096 s for phonation. The interaction is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

that the male's phonation was significantly longer than those of females in the songs with 3 singers. Running the models for each sex, we found that the phonation of both sexes was positively influenced by the contribution, the wpIOI, and the nDPs. We found that song duration had a negative effect on the females' phonation, and that the bpIOI had a negative effect on the males' phonation (null vs. full, females: $\chi^2 = 324.649$, df $= 7$, $P < 0.001$; males: $\chi^2 = 301,978$, df $= 7$, $P < 0.001$; see Table 5).

Discussion

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

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

The singing rhythm, as defined by bpIOI, appeared influenced by different parameters between the sexes. In fact, in both sexes bpIOI is influenced by the number of DPs and song duration, but for males it also depends on phonation and wpIOI. We should expect that adult males should show a less variable input to the song than those of
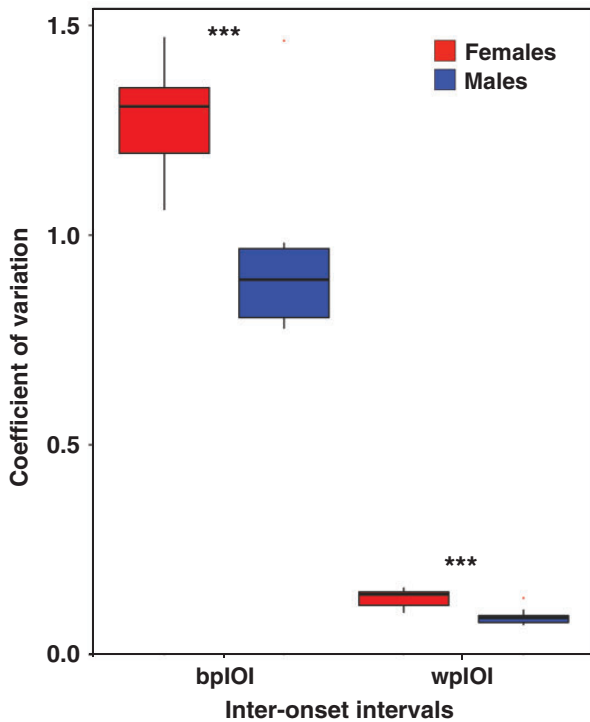
Figure 3. Boxplot of the CV of bpIOI and wpIOI in the sexes (males in blue and females in red). The values shown are calculated from the average individual means. Paired *t*-test significance at $P < 0.001$ is denoted by ***.

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 between 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 reduction in the number of bouts. Our findings are also in agreement with the results of recent studies on white-handed gibbons *Hylobates lar*, where female contribution varied in length. However, in the white-handed gibbon male contributions were more flexible than females' ones (Terleph et al. 2018b). On the contrary, female indris appeared to change the structure of their contribution to the song more consistently than males showing more flexibility than males in song expression. We observed a mechanism in the song of the indris that may resemble a turn-taking system with short turns and rapid responses in which most of the variation lie not in the internal structure of the phrases but the timing of phrases. This fact is in agreement with the experimental studies on cotton-top tamarins (Miller et al. 2003; Egnor and Hauser 2006) and common marmoset (Roy et al. 2011), which demonstrated that New World monkeys are capable of adjusting the timing of their contribution, exhibiting a certain degree of vocal control. These studies and the data presented in this paper support the hypothesis that vocal turn-taking has an ancestral origin in the primate order (Levinson 2016).

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

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

Author Contributions

Table 4. Results of the Tukey test for the interaction including sex and chorus size (bpIOI not shown as not significant for the interaction)

Sex × 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	−3.923	**<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

Statistically significant values are indicated in bold.
M, males; F, females; (2), 2 singers; (3), 3 singers; (4), 4 singers.

Table 5. Influence of the fixed factors on male's and female's phonation and contribution

	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
wpIOI	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	7.889	**<0.001**	0.827	0.065	118.456	12.695	**<0.001**
wpIOI	−0.034	0.168	39.494	−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**

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.

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