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Sex dimorphic phrase combinatorics in the song of the indris (*Indri indri*)

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Abstract

We used a logic distance to investigate intra and inter-individual variation in the phrase combinatorics of a singing primate, the indri, which inhabits the montane rain forests of Madagascar. Indris combine long notes, short single notes, and phrases consisting of two, three, four, or five units with slightly descending frequency. We calculated the similarity across different individual songs using the Levenshtein distance. We then analyzed the degree of similarity within and between individuals and found that: i) the phrase structure of songs varied between reproductive males and females; ii) male contributions to the song are overall more similar to those of other males; iii) male contributions are more stereotyped than females' ones. The picture emerging from phrase combinatorics in the indri is in agreement with previous findings of rhythmic features and repertoire size, which also suggested that female songs are potentially more distinctive than those of males.

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Introduction

Communication between conspecifics often involves the use of vocalizations because acoustic signals allow encoding a considerable amount of information in a short time (Bradbury and Vehrencamp 2011). Animal vocal signals can be emitted in the form of short vocalizations or given in sequences of variable length (Catchpole and Slater 2008) as it happens in insects, amphibians, and mammals (Kershenbaum et al. 2016). There are several methods for investigating different levels of structural information in acoustic displays. The Levenshtein distance is a quantitative method for measuring the similarity of sequences (hereafter LD; Margoliash et al. 1991). The LD is a logical distance commonly used to quantify the difference between two strings of data (e.g., human words, sequences of visual movements or sequences of song themes; Gooskens 2004). This technique has often been used to measure similarity in human dialects (Wieling 2014), and it has been applied to animal vocal sequences, but for a very limited number of species (*Passerina cyanea*, Margoliash et al. 1991; 1994; *Phylloscopus trochilus*, Gil and Slater 2000; *Megaptera novaeangliae*: Helweg et al. 1998; Tougaard and Eriksen 2006; Garland et al. 2012). When seen in comparison with humans, animals showed a limited combinatory ability to concatenate vocal emissions in phrases, at least in the acoustic domain (Berwick et al. 2011), but the information available on the variability within a species is very little (Honda and Okanoya 1999; Takahasi et al. 2010). Moreover, few investigations on primate vocal sequences are currently available and none of them are evaluating the stereotypy of song structure between sexes using LD (Gustison et al. 2016).

Indris (*Indri indri*, Gmelin 1788) represent a distinctive species for studying vocal communication because of its rich repertoire (Maretti et al. 2010) and the impressive long-distance songs, which are unique among lemurs (Gamba et al. 2016; Torti et al. 2017). The song of the indri consists of a long series of modulated notes, organized in phrases (Gamba et al. 2011). Male and female indris within a group, including juveniles, take part in a chorusing song, which lasts 40-250 s (Maretti et al. 2010). Previous research showed that the indris can emit songs in different context and that the song can elicit different behaviors depending on their acoustic structure. Cohesion songs, emitted when the animals were dispersed in the territory, were followed by a displacement of the emitters significantly higher than that following the advertisement songs, which were usually given when the animals where in visual contact (Torti et al. 2013). Other studies have shown that male and female contributions to the song differ, both quantitatively and qualitatively, in the temporal and frequency structure of units, and repertoire size (Giacoma et al. 2010; Sorrentino et al. 2012). Sex dimorphism is also present in the modulation of the frequency of vocal emissions, in the duration of note types and the rhythmic structure of a contribution (Gamba et al. 2016; De Gregorio et al. 2018). Because group encounters in the indris are rare (Bonadonna et al. 2014; Bonadonna et al. 2017), it has been suggested that songs may play a role in finding a partner and mediate pair formation. Since previous work (De Gregorio et al. 2018) shows that females adjust their contributions in order to achieve the synchronization with males, we hypothesize that this adjustment can be also reflected in a sexually dimorphic use of phrases combination. Studies of song structure in bird duets suggested that females' song would be more acoustically variable than that of males accordingly to the territorial model of duet evolution, which is consistent with socially monogamous pairs that actively defend their territory. The active role of females of Australian magpies (*Gymnorhina tibice*) in territorial defense was correlated with a song repertoire more elaborate in comparison to that of the male. Repertoires of females were as large or larger and

115 more complex than those of males, on the level of both the syllable and the song (Brown and
116 Farabaugh 1991). Like Australian magpies, indri groups occupy non-overlapping areas in the
117 forest (Pollock 1979) and use the songs to inform neighboring groups about the occupation of a
118 territory and to actively defend the territory during group encounters (Torti et al. 2013). As the
119 indris utter advertisement and cohesion songs (Torti et al. 2013), by which they inform neighbors
120 about the sex, age, and status of singing individuals (Giacoma et al. 2010; Sorrentino et al. 2012)
121 and bring together the members of a group (Torti et al. 2013), we predicted that the female
122 contribution to the song would be structurally different than that of males.
123

Materials & Methods

Observations and recordings

127 We studied 8 groups (N_{tot}= 36 individuals) living in the Maromizaha Forest (18°56'49"S,
128 48°27'53"E). We recorded the animals between 2011 and 2017. Direction des Eaux et Forêts and
129 Madagascar National Parks provided full approval for this research (see Acknowledgements).
130 We observed a social group per week, approximatively from 6 AM to 1 PM. All recordings were
131 carried out without the use of playback stimuli, and nothing was done to modify the behavior of
132 the indris. We recorded 142 songs, consisting of duets and choruses with a maximum of five
133 individuals singing in the same song. For the analysis, we considered a total of 17 focal animals:
134 nine reproductive adult males, and eight reproductive adult females. The different number of
135 males and females is motivated by the fact that, during the study period, the reproductive male of
136 a group changed. All the songs were recorded using solid-state recorders (Olympus LS05,
137 Tascam DR-100, Tascam DR-05) at a distance comprised between 2 and 20m. We always kept
138 the visual contact with the vocalizing animals and maximized our efforts to face the focal
139 animals during the emission of the song. Sequences from multiple years were present in the
140 sample, but the songs were all labeled as advertisement songs and were recorded in the same
141 context (Torti et al. 2013). Using the focal animal sampling technique (Altmann 1974), we were
142 able to attribute each vocalization to its signaler. We will refer to an individual' singing within a
143 song or a chorus as an 'individual contribution.'
144

Acoustic and statistical analyses

146 We edited segments containing indri's songs using Praat 6.0.30 (Boersma and Weenink 2008)
147 and BORIS 5.1 (Friard and Gamba 2016). We saved each recorded song in a single audio file (in
148 WAV format). We saved the information related to the identity of each singer in a Praat textgrid.
149 We then labeled all the vocal units according to their belonging to a song portion (long notes or
150 descending phrases, see Torti et al. 2013 for details) and to a descending phrase (hereafter, DP;
151 see Torti et al. 2017 for details). We considered phrases consisting of two (DP2), three (DP3),
152 four (DP4), five (DP5), and six (DP6) units. This information was saved in Praat and exported to
153 a Microsoft© Excel spreadsheet (Gamba et al. 2012).
154 To understand whether there were differences in song structure between sexes, we investigated
155 the DPs combinatorics in each individual contribution. We transformed each contribution in a
156 string of labels separated by a break symbol (e.g., DP2|DP3|DP4|DP3). We obtained 142 strings
157 for females, and 119 strings for males (with an average of 13.2 songs per individual, SD = 5.91).
158 We calculated the Levenshtein distance (LD) for each pair of strings (package StringDist 0.9.4.2
159 in RStudio) because this methodology provides a robust quantitative approach for the study of

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160 animal acoustic sequences (Kershenbaum and Garland 2015). It calculates the minimum number
161 of necessary changes (insertions, deletions, and substitutions) to transform one string into
162 another (Kohonen 1985). We obtained a squared matrix consisting of the distances between each
163 pair of strings. We then averaged LDs to calculate within- and between-individual means and to
164 investigate whether females and males differed in their degree of variation. For this purpose, we
165 ran Mantel tests (9999 randomizations) using a matrix featuring the average individual means
166 against a model matrix consisting of 0 when the corresponding individuals were of the same sex
167 (Krull et al. 2012), and 1 when they were opposite sexes (package *vegan* in RStudio). When
168 investigating differences at the group level or within-sex, we used the non-parametric paired
169 samples Wilcoxon test to compare the average individual LDs of each member of a pair or the
170 within- versus between-individual LDs. In the case of such a small sample size, the Mantel test is
171 not recommended (Legendre and Fortin 1989). Only for the Wilcoxon test, the group in which
172 the male changed was entered twice, considering the two pairs as different groups.
173

Results and Discussion

175 We analyzed 260 individual contributions consisting of a total of 2018 phrases. We obtained 77
176 $\pm$ 21 phrases per male and 78 $\pm$ 23 phrases per female. We found that average phrase duration
177 was 1.285 s (range: 0.380 - 3.000 s). The number of phrases in the individual song ranged
178 between 2 and 27 phrases.
179 We found a significant difference between the LDs calculated for males and females, where
180 females showed higher average individual means than males (Mantel test: $r = 0.167$, p-value =
181 0.002). In all groups, the females had higher LDs (LD = 6.497 + 1.674) than those of males (LD
182 = 3.946 + 0.814) showing that female contribution to the song was less stereotyped (Fig. 1,
183 Wilcoxon paired test: $V = 0$, df = 7; p-value = 0.007813). Both females and males showed a
184 higher variability at the between-individual ($LD_{females} = 7.386 \pm 0.709$, $LD_{males} = 4.885 \pm 0.325$)
185 than at the within-individual level (Fig. 1), except for the females of groups 4 and 8. Overall, we
186 found a significant difference between within- and between-individual LDs (Wilcoxon paired
187 test: $V = 0$, df = 7; p-value = 0.007813).
188
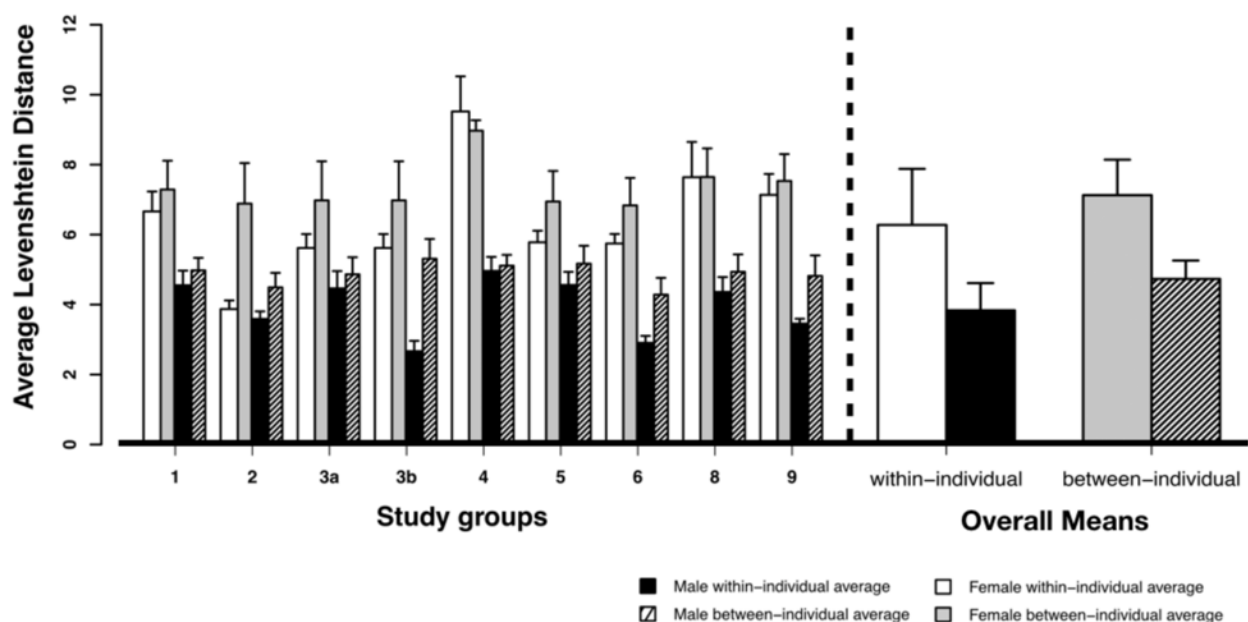
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191 **Figure 1: Comparison of Average Levenshtein Distance among sexes and individuals, in the nine studied groups.**
192 Bar plot describing the individual and overall degree of stereotypy and variability expressed by the average
193 Levenshtein Distances (LDs). Within-individual LDs are reported for females (white bars) and males (black bars), as
194 well as between-individual LDs (grey bars for females, striped bars for males). Group 3 is reported twice because
195 the male of the reproductive pairs changed in 2014. Capped lines represent Standard Deviation
196
197 We found support for our prediction that the phrase structure of songs varied between
198 reproductive males and females. The LDs showed that the between-individual stereotypy of male
199 contributions is much higher than females' one. Males, therefore, appeared to produce songs that
200 are overall more similar to those of other males and showing higher stereotypy when compared
201 to females. In agreement with previous studies that reported sexual dimorphism in the overall
202 timing and repertoire size (Giacoma et al. 2010), and the frequency modulation, duration and the
203 rhythm (Gamba et al. 2016; Torti et al. 2017, De Gregorio et al. 2019), we found that male and
204 female indris also differed in the phrase combinatorics of their songs. This result is in line with
205 the hypothesis that female components of the song were more complex than that of males,
206 suggesting that singing for females may serve to advertise the mated status of their partner and
207 prevent extra-pair copulations and male desertion, as it happens in birds (Levin 1996). In
208 agreement with previous findings on the different role of males and females during the song
209 (Giacoma et al. 2010), we found that female song is potentially more distinctive than that of
210 males. We expanded the findings of Sorrentino and colleagues (2012) showing that females not
211 only have a broader repertoire of units, but they also emit descending phrases that we did not
212 observe in males (e.g., descending phrases of six units).
213 These results are in agreement with previous finding on birds (Brown and Farabaugh 1991)
214 confirming that in those species in which females are involved in territorial defense, their
215 repertoires are as large or larger than those of males, on the level of both units and phrases. In
216 support of the higher variability in female song structure, there is the recent evidence that
217 genetics plays a critical role in determining the characteristics of DPs in males, whereas it may
218 have a lesser impact on female songs (Torti et al. 2017). A more variable song structure may, in
219 fact, add up to a more flexible structuring of the phrase notes, but further investigations are
220 needed.
221 This work also expands on and complements previous studies on humpback whales (Helweg et
222 al. 1998; Tougaard and Eriksen 2006), showing that the Levenshtein distance is simple,
223 efficiently computable and highly applicable to any behavioral data that are produced in a
224 sequence. Our results confirmed that the Levenshtein distance method is a simple but powerful
225 technique that can be applied to assess stereotypy or divergence between sexes.
226

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